Office. An appeal brief was originally due May 27, 2003. Applicants hereby petition for a one-month extension for filing an appeal brief. The fee for a one-month extension of time for a small entity is FIFTY-FIVE DOLLARS ($55.00). A check for the amount of FOUR HUNDRED AND THIRTY DOLLARS ($430.00) is enclosed, which amount includes the $55.00 extension fee and $375.00 fee for filing the accompanying Request for Continued Examination (RCE) being submitted in lieu of an appeal brief. With a one-month extension of time, an appeal brief is now due June 27, 2003. Accordingly, this application is pending today, and the accompanying RCE is being timely filed.

Please amend the subject application as follows:

In the specification:

Please amend the specification under the provisions of 37 C.F.R. §1.121(c) as follows. A marked-up version of the amended specification is attached hereto as **Exhibit A.**

Please delete the paragraphs beginning on page 4, line 14 and ending on page 4, line 28, and insert the following paragraphs:

-- **Figures 4A, 4B, 4C and 4D.** ABAD expression in Tg PD-ABAD mice (+) compared with nontransgenic littermate controls (-). Figure 4A (Northern) and Figure 4B (Western) analysis of homogenates of cerebral cortex. Equal amounts of RNA (note approximately equal intensity of 28S ribosomal RNA band on the ethidium bromide stained gel) and protein were loaded in each lane. Figures 4C-4D show immunohistochemical identification of ABAD in cerebral cortex from a Tg PD-ABAD mouse (Figure 4C) and a nontransgenic littermate control (Figure 4D).

B1 Cont

Figure 5. ABAD expression in brain subregions of Tg PD-ABAD mice compared with nontransgenic littermate controls (nonTg). Immunoblotting was performed protein extracts of brain homogenates derived from the indicated brain subregion. --

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Please delete the paragraph beginning on page 5, line 25 and ending on page 6, line 6, and insert the following paragraph:

-- **Figures 7A-7D.** Induction of stroke in Tg PD-ABAD mice. Figures 7A-7B. Tg PD-ABAD mice and nonTg littermates were subjected to middle cerebral artery occlusion and were evaluated 24 hrs after the ischemic insult to determine neurologic deficit score (Figure 7B), and, following sacrifice, infarct volume (Figure 7A). Figures 7C-7D. At the same time point, cerebral cortex was harvested to determine ATP, lactate and ß-hydroxybutyrate (BHB) levels determined on extracts of whole brains (from animals subjected to the stroke procedure 24 hrs previously) from Tg PD-ABAD or nonTg control mice (N=5, in each case). Data is reported as the mean ± SD ($P<0.04$ for ATP and $P<0.03$ for lactate). Transient middle cerebral artery occlusion model of stroke in mice: comparison of infarct volume in Tg PD-ABAD and nontransgenic littermate controls (nonTg). *$P<0.05$. --

B2

Please delete the paragraphs beginning on page 6, line 27 and ending on page 7, line 11, and insert the following paragraphs:

-- **Figures 11A-11B.** Semiquantitative analysis of synaptophysin immunoreactivity in hippocampus of Tg PD-ABAD/hAPP, Tg PD-ABAD, Tg hAPP, and nontransgenic

B3

littermate control mice at 4 months of age.

Figures 12A1, 12A2, 12A3, 12A4, and 12B. Increased expression of activated caspase-3 in cerebral cortex from Tg PD-ABAD/hAPP mice. Figures 12A1-12A4, immunostaining for activated caspase-3. Figure 12B, quantitation of immunocytochemical results from multiple fields of all mice in each of the experimental groups. Scale bar, 10 μm.

B3 cont

Figures 13A-13B. Northern analysis (Figure 13A) and immunoblotting (Figure 13B) of E16 cortical neuron cultures with ^{32}P-labelled human ABAD cDNA (Figure 13A) or anti-human ABAD IgG (Figure 13B). (+) indicates neurons obtained from Tg PD-ABAD mice and (-) indicates neurons are from nontransgenic littermate controls. --

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Please delete the paragraph beginning on page 17, line 17, and insert the following paragraph:

-- The following procedure is carried out: Introduction of ABAD into neurons or other cells (we did this also in cultured COS cells as described hereinbelow in the Examples), increases their resistance to metabolic stress as represented by an ischemic microenvironment, excitotoxic stress or nutritional stress (decreased glucose). We have recently obtained data that shows in a kainate model of brain injury, neuronal loss is less in these ABAD transgenic mice.--

B4

Please delete the paragraph beginning on page 49, line 10, and insert the following paragraph:

-- In each case, the *in vitro* and *in vivo* systems based on

B5

BS cont

Tg PD-ABAD mice or cells derived from them are ideal for studying ABAD inhibitors, as well as for dissecting contributions of ABAD to physiologic/pathophysiologic outcomes. --

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Please delete the paragraphs beginning on page 63, line 20 and ending on page 65, line 20, and insert the following paragraphs:

B6 cont

-- ABAD metabolism of ß-hydroxybutyrate. The broad enzymatic properties of ABAD as an oxidoreductase suggested that it might facilitate cellular utilization of ketone bodies, such as D-ß-hydroxybutyrate, a major energetic substrate during nutritional deprivation *in vivo*. First, we tested DL-ß-hydroxybutyryl-CoA, a known substrate of bovine liver-derived hydroxyacyl-CoA type II (HADH II)/ABAD (17), with our purified *E. Coli*-derived human recombinant ABAD. The latter result, obtained with a racemic DL mixture of ß-hydroxybutyryl CoA, was similar to that observed previously with the bovine liver HADH II (17). Using the same preparation of recombinant ABAD, we then studied D-ß-hydroxybutyrate. In a purified system, ABAD is clearly more effective with ß-hydroxybutyryl-CoA as a substrate (presumably this is the L-form, which is an intermediate in the fatty acid ß-oxidation pathway in mitochondria). However, depending on physiological conditions, certain substrates may turn out to be more abundant, such as D-ß-hydroxybutyrate during periods of starvation when levels of ketone bodies are elevated and, thus, could become relevant. In fact, plasma levels of ß-hydroxybutyrate are reported to reach the millimolar range in animals and humans subject to nutritional deprivation (21-23). Furthermore, ß-hydroxybutyryl CoA

generated by acyl CoA dehydrogenase is another likely substrate of ABAD in a cellular milieu rich in ß-hydroxybutyrate. Thus, ABAD would appear to have the potential to be pivotal for enhancing metabolism of ß-hydroxybutyrate.

Characterization of COS cells stably-transfected to overexpress ABAD. COS cells provided a useful model to test our concept that ABAD modulated the cellular response to nutritional stress because of their low endogenous expression of ABAD; low levels of mRNA were present and no antigen was detectable in lysates of wild-type COS cells. Following stable transfection with either pcDNA3 alone (vector), pcDNA3/wtABAD (encoding wild-type ABAD) or pcDNA3/mutABAD (encoding a mutant form of ABAD devoid of enzymatic activity; 14), cells were plated at limiting dilution and clones were prepared. Three types of clones were established, those expressing vector alone (COS/vector), wild-type ABAD (COS/wtABAD) and mutant ABAD (COS/mutABAD). Studies were performed with three representative clones of each type of stably-transfected COS cell. Whereas COS/vector cells displayed low levels of ABAD transcripts and antigen, comparable to control COS cells, COS/wtABAD cells showed high levels of ABAD mRNA and antigen. Subcellular fractionation studies on COS/wtABAD cells demonstrated the presence of ABAD both in fractions 1-2 enriched for the endoplasmic reticulum marker GRP78 and in the mitochondrial pellet (fraction 6) containing cytochrome c. Similar experiments performed with COS/mutABAD stable transfectants displayed high levels of ABAD transcripts and antigen in a distribution analogous to that seen in COS/wtABAD cells. These data indicated that in COS/ABAD stable transfectants, the enzyme is present at the

B6 cont

B6 cont

same sites previously observed in cells endogenously expressing ABAD or those transiently transfected to overexpress ABAD (13,14,16,19).--

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Please delete the paragraph beginning on page 72, line 10 and ending on page 73, line 6, and insert the following paragraph:

B7

Increased expression of ABAD in human brain following cerebral infarction and in response to experimentally-induced cerebral ischemia suggests that induction of ABAD might subserve normal protective mechanisms. In view of the complexities of cellular metabolic pathways, it was necessary to prove that ABAD could promote metabolic homeostasis in response to nutritional deprivation. ABAD-transfected COS cells displayed increased energy charge and flux of acetyl-CoA through the TCA cycle in medium containing ß-hydroxybutyrate compared with controls in which the active site of ABAD was mutationally inactivated. Enhanced metabolic homeostasis was reflected by maintenance of MTT reduction and morphologic phenotype in ABAD-transfected COS cells. Similarly, transgenic mice overexpressing ABAD in cortical neurons demonstrated increased flux of acetyl-CoA through the TCA cycle following ß-hydroxybutyrate infusion compared with nontransgenic littermates. However, increased basal levels of ATP (and energy charge; data not shown) in brains of Tg PD-ABAD mice, even before nutritional stress, was unexpected, and suggests a more general protective potential of ABAD in response to a range of environmental challenges. This apparent increase in the overall energy charge in the presence of ABAD, implies that the enzyme may render neurons metabolically more stable and, thus, less susceptible to fluctuations in substrate availability. --

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